Questions, Comments: Small Mammal and Amphibian DQOs

Remedium appreciates the opportunity to again review the small mammal and amphibian DQOs. We continue to have several questions and also offer several important comments that should be addressed in the final Phase III SAP DQOs.

1. Small Mammal DQO

- Possible tissue submittal for LA analysis. Currently text does not address how this will be done, though it is a secondary goal of the study. Requires explanation on how this will be done should it be needed. Would require that part of each tissue be reserved for possible LA analysis (or for lungs and adrenals, one each reserved).

EPA Response: The attached SOP, "Small Mammal Collection and Processing" includes details on tissue collection and preservation for possible tissue burden analysis. The SOP calls for half of each tissue to be preserved for histopathology and half to be preserved for asbestos tissue burden analysis. We request that Remedium have this SOP reviewed by the histopathologist who will be performing the tissue examination and EPA will ensure the analytical laboratory reviews it as well and will coordinate discussions between the laboratory and the histopathologist as necessary. EPA will make any modifications to the SOP that may be needed to accurately reflect the procedures that will be used. Alternatively, if there is an existing Parametrix SOP that describes the procedures, EPA is willing to consider it for the Phase III program. Please submit the proposed SOP to EPA for review. The SOP will be final when signed by EPA.

- Given how small the tissues noted above may be the laboratory must be able to detect LA based on very small tissue weights.

EPA Response: We will confirm that the required analysis can be performed on the size tissues likely to be sent to the laboratory.

- Section 4.2.5.2, second paragraph, second sentence. Gross deformities? No data to support that LA causes gross deformities. This seems to be a hold over from the amphibian DQO or an anomaly that should be removed.

EPA Response: We believe that any gross deformities are important to record if they are observed. The text will stay as it's written.

- At the Denver BTAG there was discussion of one duff composite sample being collected each at the OU3 and the reference area. This DQO does not discuss any duff samples at all. Though we support that duff samples are not needed, can EPA confirm that duff sampling is now off the table for both OU3 and the reference area so it is clear this is not an omission?

EPA Response: No duff samples will be collected as part of the Phase III sampling program. Although this was discussed at the February 2009 BTAG meeting in Denver, EPA decided not to include duff sampling since it's not needed for the objectives of the small mammal investigation. That is, we are not attempting to establish a relationship between observations of adverse effects and exposure to duff. We may want to confirm exposure if a significant difference in adverse effects is observed in the impacted area compared to the reference area, but we will rely on tissue burden information for this objective. Additionally, the collection of a composite sample over the trapping area will be impractical due to the large sample mass.

- Conversations with histopathologists and a cursory review of the literature both indicate that there are no pathologies associated with CO2 asphyxiation. Therefore, we are rather insistent that CO2 asphyxiation (then followed by cervical dislocation) be noted in this DQO rather than simply "cervical dislocation". Two reasons for this: first, Institutional Animal Care and Use Committee (IACUC) requirements do not permit cervical dislocation alone without prior anesthesia for adult rodents (permissible for juvenile/neonates), and secondly, CO2 asphyxiation renders the animal moribund and easier for those of us processing the animal to handle. For adult rodents, IACUC guidelines discuss using a combination of CO2 asphyxiation (first), followed by cervical dislocation on the moribund animal to ensure animals do not recover from the asphyxiation. This is the planned method for euthanasia of the adult rodents collected. We require that this language be put into the small mammal DQO as this will be the procedure followed for animal euthanasia prior to necropsy.

EPA Response: The attached SOP and the SAP have been modified to reflect CO2 asphyxiation followed by cervical dislocation as the planned method for euthanasia of the adult rodents collected.

- Please correct the DQO to note that the animal will not be skinned (there is no technical, scientific or toxicological reason for doing so). Following necropsy the animal will be wetted with a slightly soapy solution to control release of fur into the open body cavity as well as to control airborne release of any particles/fibers from the animals' fur.

EPA Response: The SAP has been modified to reflect that the mammals will not be skinned.

2. Amphibian DQO

- The "severity" of a malformation that could affect growth, survival and reproduction is identified as a measurement endpoint but the decision criteria to support it are not provided (and should be for transparency). Severity is a subjective term and the decision criteria for assessing that a malformation is severe enough to impact survival, growth and/or reproduction must be specified.

EPA Response: The text will be modified to reflect that study measurement endpoints are incidence of malformations that could affect growth, survival, and reproduction and do not include judgment of severity.

- Tank cleaning requires clarification. What is EPA's thinking on this relative to ensuring fibers are not lost? This is an important detail to understand in this DQO since it can affect fiber concentration.

EPA Response: The testing laboratory will be required to submit a study protocol to EPA that will include details of the tank cleaning. Any requirements to mitigate potential fiber loss during cleaning will be included in the protocol. The BTAG will have an opportunity to review the protocol.

- The stock solution (Attachment G) protocol does not appear to be correct and should be revisited. The final concentration listed in this protocol "5,000 to 20,000 MFL" is actually unknown since it requires verification by a lab before it is known. Regardless, if this specified concentration range is supposed to reasonably represent the final stock solution concentration then the concentration is too high and requires another dilution to bring it down to 100 MFL. This final dilution step is absent from the protocol. <u>The final stock solution should be at the maximum test concentration of 100 MFL when provided to the testing laboratories</u> so that the amphibian test can be run with it directly and so that serial dilutions can be made for the rainbow trout tests. Therefore, we suggest that this protocol be revisited and corrected. Finally, the protocol should specify that EPA will prepare and undertake the confirmation testing of the stock solutions and then provide the final stock solution (at 100 MFL concentration) to each lab to utilize in their testing regimes.

EPA Response: Attachment G is being revised with input from the USGS, the analytical laboratories, and the testing laboratories. The protocol for preparing spiked water will be included in the study protocols to be prepared by the testing laboratories and submitted to EPA.

- The general well being, motility and activity level of animals with obvious malformations relative to other animals lacking malformations should be recorded regularly as well (not just the "occurrence of malformations").

EPA Response: The study protocol to be submitted by the laboratory will contain the details of observations that will be recorded.

- Necropsy: is the testing laboratory performing the necropsies and the histology? The protocol seems to imply this. The DQO should be clarified.

EPA Response: The study protocol to be submitted by the laboratory will contain these details.

- Table 4-8, Bin C should say >1%, not ?1%.

EPA Response: The text has been corrected.